

Prevalence of pathogenic bacteria in dental prostheses at Zawia private clinics

Ali Milad¹ , Najeeb Shebani² , Zakaria Khmaj³ , Hadeel Izeit⁴ , Tarek Dokhan^{2*} , Mohammed Gendel⁵

¹Department of Orthodontics, Faculty of Dentistry, University of Zawia, Libya.

²Department of Dental Technology, Faculty of Medical Technology, University of Zawia, Libya.

³Department of Orthodontics and Dentofacial Orthopedics, Belarusian Medical Academy of Post-Graduate Education, Minsk, Belarus.

⁴Department of biological sciences/Biomedical sciences, Libyan Academy Misrata, Libya.

⁵National Center for Disease Control, Zawia branch, Libya.

ARTICLE INFO

Corresponding Author:

Tarek Dokhan

Department of Dental
Technology, Faculty of
Medical Technology,
University of Zawia, Libya.

Email: t.dokhan@zu.edu.ly

Received:

03 July 2023

Received in revised form:

07 August 2023

Accepted:

15 August 2023

ABSTRACT

Purpose: The oral cavity's dental prosthesis may serve as a reservoir for bacteria that are connected to systemic infections. For this purpose, this study aimed to investigate and identify the bacteria pathogenic strains in the oral cavity and their prevalence in dentures, fixed prosthodontics, and orthodontic appliances wearers.

Materials and Methods: One hundred samples of swabs were collected from private dental clinics in Zawia, Libya. These samples were taken from male and female patients who wear dental prosthesis. The swabs were cultured, and the bacterial strains were identified.

Results: Forty-three percent of samples were pathogenic, which showed the following strains: *K. pneumoniae*, *S. aureus*, *E. cloacae*, *P. aeruginosa*, *Pneumococcus*, *E. coli*, *A. humani*, and *S. pyogenes* strains were found, and they were in males more than females. Moreover, the results showed that most of these strains existed on orthodontic appliances.

Conclusion: The prevalence of oral pathogens is higher in males than females (50% and 38.7%, respectively). Most pathogenic bacteria were found on the surface of orthodontic appliances more than the other dental prosthesis. (*Open J Biomed Res* 2023;2:3-7.)

Keywords

Dental prosthesis, oral pathogens, antibiotic resistance, bacteria, orthodontic appliances.

AUDIOABSTRACT



INTRODUCTION

In the human oral cavity, there is a large and diverse microbial population that includes bacteria and fungi.¹ The majority of the bacteria in the human mouth are anaerobic gram-negative and gram-positive bacteria. Since this bacteria plays an essential role in preventing the colonization of pathogenic microbiota, any changes to this normal flora have an impact on dental health.^{1,2} The main entrance to the human body is through the mouth cavity, which is a complex and dynamic environment.^{3,4} Over 1000 species of oral microbiota have been identified by many studies.^{5,6} The oral microbiota of humans has been used as a model system for research on multispecies microbial communities,⁷⁻⁹ ever since Antonie van Leeuwenhoek first identified bacteria from the oral cavity in the 18th century.¹⁰

Dentures may affect the environment in the oral cavity due to lowered pH levels, reduced saliva flow on tissue surfaces, and a lack of mechanical tongue cleansing.¹¹ Moreover, the chance of microbe accumulation increases due to the roughness of the acrylic resin, which encourages plaque formation on denture surfaces.¹²

Adults and the elderly use complete or partial dentures in response to a variety of disorders that affect tooth extraction.^{13,14} Denture wearers should be aware that dentures can act as a breeding ground for oral bacteria and the growth of halitosis.^{15,16} The microbial community of the mouth undergoes some modifications when removable dental prostheses are used. This circumstance can occasionally result in stomatitis linked to dentures or dental prosthetics.¹⁷

MATERIALS AND METHODS

One hundred patient' participants were chosen for swab collection for this study, all those patients were wearing dental prostheses, which included fixed and removable prostheses as well as orthodontic appliances. The samples were collected from the local dental clinics in Zawia city. Any random patient visiting a dental clinic, excluding those under the age of five or those suffering from viral infections, met the study's inclusion criteria. Swabs were obtained and sent to the ALFA medical laboratory in Zawia, Libya. There, they were incubated overnight at 37°C in a shaking incubator. By inoculating the material on nutritional agar media plates which included beef extract 0.3%, peptone 0.5%, and agar 1.5% in water.

For this purpose, a sterilized inoculating loop was used to streak samples onto nutrient agar plates. After being inoculated, the nutrient agar plates underwent a 24h at 37°C thermal incubator incubation.

Using a gram staining kit, all pure bacterial isolates were initially processed. Under a microscope with a 100x magnification, the prepared slides were examined. Based on the colors and cell arrangement of the bacterium, observations were made.

To distinguish between bacterial strains, various biochemical assays were carried out after gram staining and microscopy. Basic biochemical (Api-20E) assays like the Oxidase, Catalase test (H₂O₂), and Coagulase test (EDTA) were used to distinguish between bacterial strains, and *Staphylococcus* colonies were also cultured on mannitol salt agar.

The findings of all samples were compared between the two groups using the paired t-test. And the χ^2 test were used for categorical variables of more than two groups. The significant difference between the groups was indicated by a P-value of 0.05.

RESULTS

Bacteria Strain Identification and Data Distribution of Gender:

This study included 100 samples of oral cavity swabs from dental patients at clinics located in Zawia, Libya, gathered from people aged from 12 to 58 years old. The samples were taken from both genders: 38 males (38% of the total) and 62 females (62% of the total), (P= 0.08) as shown in [Figure 1](#).

Among 100 samples collected ([Fig. 2](#)), 43 samples (43%) showed growth on the cultured media, and the majority of pathogenic bacteria were *K. pneumoniae*

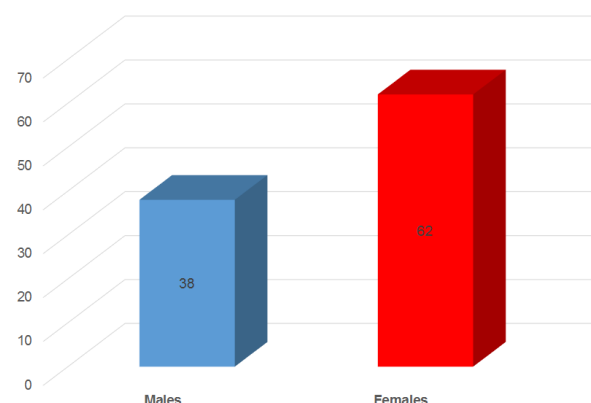


Fig 1. Gender distribution.

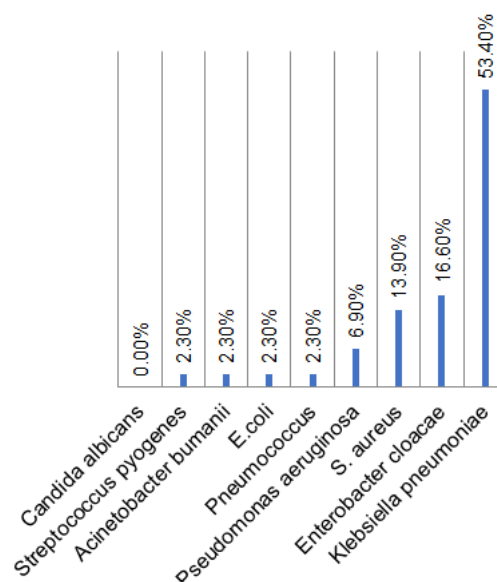


Fig 2. Bacterial strains identified in the oral cavity.

(53.4%), followed by *E. cloacae* (16.6%), *S. aureus* (13.9%), and *P. aeruginosa* (6.9%) ([Table 1](#)).

Apart from these significant findings, from the total 100 samples, 43 % showed growth on the cultured media; it showed significant differences at ages 13-35 years with 34 (44.2%) samples. The other 57% of samples did not show any bacterial growth ([Table 2](#)).

Due to the different age groups used in these studies, it is difficult to compare our findings to those of previous studies. Microorganism isolation was highest among those aged 13 to 65 with risk factors such as smoking, diabetes, and infrequent brushing of the teeth ([Fig. 3](#)).

Table 1. Number and types of strains.

Name of bacteria	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Pneumococcus</i>	<i>E.coli</i>	<i>Acinetobacter bumanii</i>	<i>Streptococcus pyogenes</i>	<i>Candida albicans</i>	Total
Number	23	7	6	3	1	1	1	1	0	43
Percentages	53.4%	16.6%	13.9%	6.9%	2.3%	2.3%	2.3%	2.3%	0.0%	100%

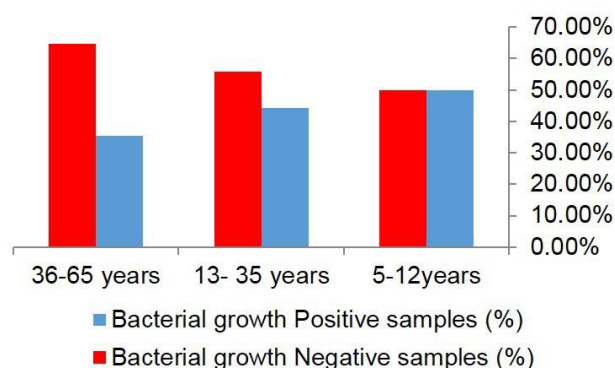
Table 2. Distribution of positive and negative bacterial growth, and age groups.

Age Groups	Bacterial growth		Total samples (%), p-value=0.07
	Positive samples (%)	Negative samples (%)	
5-12years	3 (50.0%)	3 (50.0%)	6 (6%),
13- 35 years	34 (44.2%)	43 (55.8%)	77 (77%)
36-65 years	6 (35.3%)	11 (64.7%)	17 (17%)
Total	43 (7%)	57 (7%)	100%

Presence of Bacteria

Pneumococcus, *A. bumanii*, and *S. pyogenes* were present only in female samples. While *E. coli* was present only in male patients, *S. aureus* was detected in female samples more than male samples. while *K. pneumoniae* was presented almost the same in male and female cases (12 and 11, respectively).

Table 3 shows that the orthodontic appliances were the most commonly found bacteria strain, with 22 samples of *K. pneumonia*, 5 samples of *S. aureus*, 6 samples of *E. cloacae*, and one sample for these strains, *P.aeruginosa*, *P.neumococcus*, *E. coli*, *A. bumanii*, and *S. pyogenes*. While, the microbiological composition of the white filling cases are 2 samples of *K. pneumonia* and 1 samples of *E. coli*. Various degrees of contamination of gingival sulcus with microorganisms were noted at Gingiva, Amalgam, and PFM Bridge constructions with

**Fig 3.** Distribution of positive and negative bacterial growth with age groups.

one *P. aeruginosa* sample in Gingiva. However, In the Amalgam sample showed 3 samples of *K. pneumonia*, 2 *S. aureus*, and 1 *P. aeruginosa*. And bridge constructions have just 1 sample containing *E. cloacae* bacteria.

Table 3. Number and types of strains related to location in the oral cavity.

<i>C. albicans</i>	<i>S. pyogenes</i>	<i>A. bumanii</i>	<i>E. coli</i>	<i>Pneumococcus</i>	<i>P. aeruginosa</i>	<i>E. cloacae</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	
0	1	1	1	1	1	6	5	22	Orthodontic Appliance
0	0	0	1	0	0	0	0	2	White Filling
0	0	0	0	0	1	0	0	0	Gingiva
0	0	0	0	0	1	0	2	3	Amalgam
0	0	0	0	0	0	1	0	0	PFM Bridge

DISCUSSION

This study analyzed the prevalence of microorganisms in dental prosthesis at dental private clinics. Our meta-analysis consisted of 100 samples from dental patients who were wearing this prosthesis.

Healthy individuals with or without teeth may have yeast and bacteria colonizing their mouths that cohabit in a commensal relationship. Numerous circumstances

may cause the number of bacteria in the oral cavity to rise, which might encourage colonization and allow them to develop into harmful organisms. Denture wearing and poor denture cleanliness are among the predisposing factors.¹⁸

A study conducted by Dag Istan S et al.¹⁸ confirmed the presence of *Candida spp.* and *S. aureus* on 90 patients' maxillary full dentures. The majority of

the microorganisms on the dentures were discovered to be *Candida* species (65.5%), followed by *Strep* mutans (53.3%) and *S. aureus* (34.4%). These findings support those made by Baena-Monroy et al.¹⁹, who showed that *C. albicans*, *S. aureus*, and *Strep*. mutans were present on the inner surface of complete dentures. In their investigation, *S. aureus* and *Strep*. mutans were isolated from 49.5% of the dentures, while *C. albicans* were found in 66.7% of them. Comparably, another study discovered that 64.2% of people who wear dentures have *Candida* spp.²⁰

There aren't many studies looking at *P. gingivalis* colonization in adolescents using fixed orthodontic appliances for extended periods of time (up to six months). According to prior studies, several periodontal bacteria significantly increased after 6 months of fixed appliance therapy but decreased after 12 months of orthodontic therapy.²¹ According to Ristic et al., the detection rate of *P. gingivalis* changed with changes in the gingiva index and increased from baseline to three months after bonding before declining at six months.²²

The association between oral bacteria and orthodontic therapy has been the subject of numerous studies using traditional techniques, such as culturing, and these studies have concentrated on certain bacterial species. For instance, with fixed orthodontic appliances, increases in the prevalence of tooth caries and *S. mutans* bacterial counts were observed.^{23,24} The positioning of orthodontic appliances also had an impact on clinical indicators and the colonization of periodontal pathogenic bacteria like *P. gingivalis*, *P. intermedia*, *P. nigrescens*, and *F. nucleatum*.²⁵⁻²⁷

In another study done by Derafshi R et al.²⁸, the difference in the salivary microbial population between persons wearing removable dentures and the control group was examined, indicating a variety of pathogens in removable dentures, including *E. coli*, *K. pneumoniae*, and *E. aerogenes*. Gram-negative bacteria belonging to the *Enterobacteriaceae* family and nonfermenting bacteria were significantly more prevalent in the case group than in the control group. Potentially harmful microorganisms, such as gram-negative bacilli from the *Acinetobacter*, *Pseudomonas*, *Moraxella* *Micrococcus*, and *Alcaligenes* species, have been identified as causes of contamination in industrial dentistry laboratories.^{29,30}

Pathogens that cause pneumonia from inhalation can be significantly harmed by dentures.³¹ It has been demonstrated that some rare microbes can be isolated from dentures, including *P. aeruginosa*, *S. pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *E. coli*, and *K. pneumoniae*.³²

The eradication of the germs that cause this condition is necessary for an endodontic treatment to be successful. Although it has been demonstrated that chemo-mechanical disinfection is successful in lowering intracanal bacteria.³³

Our finding indicates that wearing dental prosthesis can be considered a risk factor for colonization by different species of microbiota. The results of our experiments showed that there are pathogenic bacteria such as *K. pneumoniae*, *S. aureus*, *E. cloacae*, *P. aeruginosa*, *Pneumococcus*, *E. coli*, *A. bumanii*, and *S. pyogenes* by 43%. However, no *C. albicans* was detected in this study. It's important to note that, at 50% and 38.7%, respectively, males were found to have a considerably higher prevalence of these germs than females. *K. pneumoniae* was present in 53.4% of the total pathogenic samples, while *E. cloacae* was 16.6%, *S. aureus* 13.9%, *P. aeruginosa* 6.9%, *Pneumococcus* 2.3%, *E. coli* 2.3%, *A. bumanii* 2.3%, and *S. pyogenes* 2.3%.

Additional studies are advised to investigate the oral biofilm in the general population, including those in Zawia, Libya, who are not currently receiving orthodontic and prosthetic treatment. The assessments should be made with the intent of evaluating the biological impact of the bacterial species on the oral cavity, their frequency in the development of oral diseases, and potential techniques for bacterial prevention and management.

CONCLUSION

This study verified the prevalence of *K. pneumoniae*, *S. aureus*, *E. cloacae*, *P. aeruginosa*, *Pneumococcus*, *E. coli*, *A. bumanii*, and *S. pyogenes* on dental prosthesis surfaces, especially on orthodontic appliances.

These results highlight the significance of practicing good oral hygiene while receiving orthodontic treatment, as well as the necessity of routinely cleaning dental prosthesis to reduce the risk of bacterial colonization and potential oral health issues.

REFERENCES

1. Samaranayake L, Matsubara VH. Normal oral ora and the oral ecosystem. *Dent Clin North Am.* 2017;61:199-215.
2. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Denying the normal bacterial ora of the oral cavity. *J Clin Microbiol.* 2005;43:5721-5732.
3. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. *Oral Dis.* 2012;18:109-120.
4. Craig SJC, Blankenberg D, Parodi ACL. Child weight gain trajectories are linked to oral microbiota composition. *Sci Rep.* 2018;8:14030.
5. Mahasneh SA, Mahasneh AM. Probiotics: a promising role in dental health. *J Dent.* 2017;5:26.

6. Gao L, Xu T, Huang G, Jiang S, GuChen YF. Oral microbiomes : more and more importance in the oral cavity and whole body. *Protein Cell*. 2018;9:488-500.
7. Kuramitsu HK, He X, Lux R, Anderson MH, Shi W. Interspecies interactions within oral microbial communities. *Microbiol Mol Biol Rev*. 2007;71:653-670.
8. Kolenbrander PE. Oral microbial communities: biofilms, interactions, and genetic systems. *Annu Rev Microbiol*. 2000;54:413-437.
9. Kolenbrander PE, Palmer RJ Jr, Periasamy S, Jakubovics NS. Oral multispecies biofilm development and the key role of cell-cell distance. *Nat Rev Microbiol*. 2010;8:471-480.
10. Gest H. The discovery of microorganisms by Robert Hooke and Antoni Van Leeuwenhoek, fellows of the Royal Society. *Notes Rec R Soc Lond*. 2004;58:187-201.
11. Budtz-Jørgensen E. Etiology, pathogenesis, therapy, and prophylaxis of oral yeast infections. *Acta Odontol Scand*. 1990;48:61-69.
12. Brill N, Tryde G, Stoltze K, El Ghamrawy EA. Ecologic changes in the oral cavity caused by removable partial dentures. *J Prosthet Dent*. 1977;38:138-148.
13. Agwu E, Ihongbe JC, Ezeonwumelu JO, Lodhi MM. Baseline burden and antimicrobial susceptibility of pathogenic bacteria recovered from oral lesions of patients with HIV/AIDS in South Western Uganda. *Oral Sci Int*. 2015;12:59-66.
14. Khorshidi H, Moaddeli MR, Golkari A, Heidari H, Rao S. The prevalence of pathologic tooth migration with respect to the severity of periodontitis. *J Int Soc Prev Community Dent*. 2016;6 Suppl 2:S122-125.
15. Wu T, He X, Lu H, Bradshaw DJ, Axe A, Loewy Z, et al. Development of in vitro denture biofilm models for halitosis related bacteria and their application in testing the efficacy of antimicrobial agents. *Open Dent J*. 2015;9:125-131.
16. De Geest S, Laleman I, Teughels W, Dekeyser C, Quirynen M. Periodontal diseases as a source of halitosis: A review of the evidence and treatment approaches for dentists and dental hygienists. *Periodontol*. 2016;71:213-227.
17. Pinke KH, Freitas P, Viera NA, Honório HM, Porto VC, Lara VS. Decreased production of proinflammatory cytokines by monocytes from individuals presenting Candida-associated denture stomatitis. *Cytokine*. 2016;77:145-151.
18. Dağistan S, Aktas AE, Caglayan F, Ayyildiz A, Bilge M. Differential diagnosis of denture-induced stomatitis, Candida, and their variations in patients using complete dentures: a clinical and mycological study. *Mycoses*. 2009;52:266-271.
19. Baena-Monroy T, Moreno-Maldonado V, Franco-Martínez F, Aldape-Barrios B, Quindós G, Sánchez-Vargas LO. Candida albicans, Staphylococcus aureus, and Streptococcus mutans colonization in patients wearing dental prosthesis. *Med Oral Patol Oral Cir Bucal*. 2005;10:E27-E29.
20. Lyon JP, da Costa SC, Totti VM, Munhoz MF, de Resende MA. Predisposing conditions for Candida spp. carriage in the oral cavity of denture wearers and individuals with natural teeth. *Can J Microbiol*. 2006;52:462-467.
21. Thornberg MJ, Riolo CS, Bayirli B, Riolo ML, Van Tubergen EA, Kulbersh R. Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy. *Am J Orthod Dentofacial Orthop*. 2009;135:95-98.
22. Ristic M, Vlahovic Svabic M, Sasic M, Zelic O. Clinical and microbiological effects of fixed orthodontic appliances on periodontal tissues in adolescents. *Orthodontics and craniofacial research*. 2007;10:187-195.
23. Lundström F, Krasse B. Caries incidence in orthodontic patients with high levels of Streptococcus mutans. *Eur J Orthod*. 1987;9:117-121.
24. Jordan C, LeBlanc DJ. Influences of orthodontic appliances on oral populations of mutans streptococci. *Oral Microbiol Immunol*. 2002;17:65-71.
25. Naranjo AA, Trivino ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. *Am J Orthod Dentofacial Orthop*. 2006;130:e217-222.
26. Ristic M, Vlahovic Svabic M, Sasic M, Zelic O. Clinical and microbiological effects of fixed orthodontic appliances on periodontal tissues in adolescents. *Orthod Craniofac Res*. 2007;10:187-195.
27. Goyal N, Shamanna PU, Varughese ST, Abraham R, Antony B, Emmatty R, et al. Effects of amine fluoride and probiotic mouthwash on levels of Porphyromonas gingivalis in orthodontic patients: A randomized controlled trial. *J Indian Soc Periodontol*. 2019;23:339-344.
28. Derafshi R, Bazargani A, Ghapanchi J, Izadi Y, Khorshidi H. Isolation and identification of nonoral pathogenic bacteria in the oral cavity of patients with removable dentures. *J Int Soc Prevent Community Dent*. 2017;7:197-201.
29. Williams HN, Falkler WA, Hasler JF, Libonati JP. The recovery and significance of nonoral opportunistic pathogenic bacteria in dental laboratory pumice. *J Prosthet Dent*. 1985;54:725-30.
30. Nair VV, Karibasappa GN, Dodamani A, Prashanth VK. Microbial contamination of removable dental prosthesis at different intervals of usage: an in vitro study. *J Indian Prosthodont Soc*. 2016;16:346-51.
31. Daniluk T, Fiedoruk K, Sciepek M, Zaremba ML, Rozkiewicz D, Cylwik-Rokicka D, et al. Aerobic bacteria in the oral cavity of patients with removable dentures. *Adv Med Sci*. 2006;51 Suppl 1:86-90.
32. Goldberg S, Cardash H, Browning H 3rd, Sahly H, Rosenberg M. Isolation of Enterobacteriaceae from the mouth and potential association with malodor. *J Dent Res*. 1997;76:1770-1775.
33. Ehlinger C, Darteville P, Zaet A, Kurashige Y, Haikel Y, Metz-Boutigue MH, et al. A New Combination with D-Cateslytin to Eradicate Root Canal Pathogens. *Int J Pept Res Ther*. 2019;25:1679-1687.